

5 restrictions on distribution of ATCC 7446 are
irrevocably removed on granting of a patent on this
application. The address of the American Type Culture
Collection is 10801 University Boulevard, Manassas,
Virginia 20110-2209.--

In the Claims:

Amend Claims 16, 18, 19, 24, and 25 as
follows.

-16-(Third amended)

A method for treatment of Pythiosis in human
patients having the disease which comprises:

- (a) providing a vaccine containing a mixture
of proteins of *Pythium insidiosum* in a sterile aqueous
5 solution, wherein the mixture of proteins consists of
(1) mixed proteins removed from disrupted cells of the
Pythium insidiosum separated from a culture medium used
to grow the cells and (2) mixed proteins removed from
the culture medium separated from the *Pythium insidiosum*
10 wherein the mixture in water has been dialyzed to remove
low molecular weight components less than 10,000 MW; and
(b) vaccinating the patient with the vaccine.

-18-(Third amended)

A method for the treatment of Pythiosis in a
mammal having the disease which comprises:

(a) providing an injectable vaccine derived from growing cells of *Pythium insidiosum* in a culture medium which comprises in a sterile aqueous solution in admixture:

(1) mixed proteins removed from disrupted cells of the *Pythium insidiosum* separated from the culture medium; and

(2) mixed proteins removed from the culture medium separated from the cells of the *Pythium insidiosum*;

wherein the admixture in water has been dialyzed to remove low molecular weight components less than 10,000 MW to produce the vaccine; and

(b) vaccinating the mammal with the vaccine.

-19-(Third amended)

The method of Claim 18 wherein the removed proteins in the admixture have been provided by growing cells of the *Pythium insidiosum* in the culture medium, then killing the cells, then separating the killed cells from the culture medium to produce a first supernatant containing the mixed proteins of step (2) and then disrupting the killed cells in sterile water and removing the disrupted cells to provide the mixed proteins of step (1) in a second supernatant, combining the first and second supernatants, precipitating the proteins, resuspending the precipitated proteins in sterile water, and dialyzing the resuspended proteins in sterile water to remove the material less than 10,000 MW.

-24-(Third Amended)

The method of Claim 19 wherein the disrupted cells are removed from the culture medium for the cells by centrifugation to provide the mixed proteins of step (1) in the second supernatant.

-25-(Third amended)

The method of Claim 19 wherein the mixed proteins from steps (1) and (2) are precipitated using acetone.